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Aspergillus similanensis sp. nov. from a marine sponge in Thailand

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Abstract —A new species of *Aspergillus* sect. *Fumigati*, *A. similanensis*, isolated from the marine sponge *Rhabdermia* sp. collected from a coral reef of the Similan Island, Phangnga province, Thailand, is described and illustrated. *Aspergillus similanensis* is characterized by production of finely reticulate ascospores with two low equatorial crests, a yellowish colony on malt extract agar, and extremely restricted growth on Czapek agar. The validation of this new species is further supported by sequence analyses of the β-tubulin, calmodulin, and actin genes.

Key words — sponge-associated fungi, phylogeny, taxonomy

Introduction

Aspergillus sect. *Fumigati* W. Gams et al. [= *Neosartorya* Malloch & Cain] is widely distributed, being reported mainly from soil, debris, and foods (Samson et al. 2007, Matsuzawa et al. 2014a,b) but recently also from marine environments, such as from marine sponges (Eamvijarn et al. 2013a). Despite the reports on their pathogenicity to humans and animals (Farrell et al. 2014, Howard 2014), *Neosartorya* species are also prolific sources of secondary metabolites with a wide range of biological activities (Eamvijarn et al. 2013b; Gomes et al. 2014; Prompanya et al. 2014, 2015).

Samson et al. (2007) revised the taxonomy of *Aspergillus* sect. *Fumigati* and *Neosartorya* based on phenotypic and molecular characteristics, describing 33 species, including 23 with teleomorphic states. Since then, several new species in *A.* sect. *Fumigati* have been reported: Eamvijarn et al. (2013b) described *A. siamensis* from coastal forest soil in Samaesarn Island, Thailand; Hubka et al. (2013) described *A. waksmanii* from New Jersey soil and *A. marvanovae* from contaminated water in the Czech Republic; Matsuzawa et al. (2014a,b) described *A. caatingaensis* and *A. pernambucoensis* from semi-desert soil in Brazil and *A. huiyaniae* from desert soil in Xinjiang, China; and Nováková et al. (2014) described *A. brevistipitatus*, *A. conversis*, and *A. wyomingensis* from reclamation site soils in Wyoming, USA.

In our ongoing search for bioactive compounds from fungi against plant pathogens, unusual isolates of *Aspergillus* sect. *Fumigati* have been isolated from the marine sponge *Rhabdermia* sp. Here, we describe and illustrate the sponge isolates as a new species, *A. similanensis*.

Materials & methods

Fungal Isolation′

The marine sponge *Rhabdermia* sp. was collected at a coral reef in the Similan Islands National Park (8°39′09″N 97°38′27″E), Phangnga province, Thailand. The sponge tissue was washed three times with sterile sea water, cut into 0.5 × 0.5 cm pieces, and placed on half potato dextrose agar (PDA) with 70% of sea water and 300 mg L^{-1} of streptomycin sulphate. The plates were incubated at 28°C for 7 days.

Hyphal tips from a pure fungal strain were transferred onto PDA slants and stored in the Culture Collection, Department of Plant Pathology, Kasetsart University, Bangkok, Thailand (KUFA) for subsequent identification and deposited in a metabolically inactive state at BIOTEC Culture Collection, Pathumthani, Thailand (BCC).

Morphological Identification

Macroscopic characteristics were observed from the colonial growth pattern (i.e., color and texture) on malt extract agar (MEA), cornmeal agar (CM), Czapek agar (CzA), oatmeal agar (OA), and Czapek yeast extract agar (CYA), after incubation at 25°C, 37°C, and 45°C for 7 days (Samson et al. 2007). Colony colors were designated according to Rayner (1970). After incubation, microscopic characteristics were thoroughly examined under a light microscope in a slide preparation using sterile distilled water as the mounting medium. Ascospore ornamentation was examined by scanning electron microscope (SEM: JEOL JSM 6400).

DNA extraction and molecular Identification

DNA was extracted from young mycelia following a modified Murray & Thompson (1980) method. Primer pairs Bt2a and Bt2b were used for β-tubulin gene amplification (Glass & Donaldson 1995), cmd5 and cmd6 for calmodulin gene amplification (Hong et al. 2005), and ACT-512F and ACT-783R for actin gene amplification (Carbone & Kohn 1999). The PCR products were sequenced using dideoxyribonucleotide chain termination method (Sanger et al. 1977) (Macrogen Inc., South Korea). DNA sequences were edited using Finch TV software. The gene sequences were aligned and phylogenetic trees were generated using Clustal X software (Thompson et al. 1997) utilizing neighbor-joining analysis (Saitou & Nei 1987). The distances between sequences were calculated based on the two-parameter model by Kimura (1980). A bootstrap was conducted with 1000 replications (Felsenstein 1985).

Taxonomy

Aspergillus similanensis Dethoup, **sp. nov.** FIGS 1–4

MYCOBANK MB810782

"*Aspergillus similanensis*" Prompanya et al., Mar. Drugs 12: 5160. 2014, nom. nud.

"*Aspergillus similanensis*" Prompanya et al., Mar. Drugs 13: 1342. 2015, nom. nud.

Differs from other *Aspergillus* species by its finely reticulate ascospores with two low equatorial crests, the production of a yellowish colony on MEA, and extremely restricted growth on CzA.

TYPE: Thailand, Phangnga Province, Similan Island, a dried culture from marine sponge, *Rhabdermia* sp., collected from a coral reef, April 2010, T. Dethoup (**Holotype**, BCC 75436; ex-type cultures KUFA 0012, KUFA 0013; GenBank KM095494, KC920701, KM202074).

ETYMOLOGY: Referring to Similan Island where the sponge was collected for fungal isolation.

Colonies on MEA growing rapidly, attaining a diameter of 35 mm in 7 days at 25°C, consisting of a floccose mycelium, producing abundant ascomata on the mycelial felt, white to creamish, margins broad, thin and plane; conidiogenesis absent; exudate none; odor lacking; reverse luteous (R12).

Colonies on CzA growing extremely restrictedly, attaining a diameter of 8–10 mm in 14 days at 25°C, producing only white mycelium covering the mycelial plug. Colonies on CYA attaining a diameter of 15–20 mm in 7 days at 25°C, velvety, floccose at the central, consisting of a compact basal felt, furrowed, margins irregular, pale luteous (R11) to sulphur yellow (R15); ascomata and conidiogenesis absent; exudates and soluble pigment absent; reverse wrinkle, slightly zonate, rust (R39).

Colonies on CM spreading broadly, attaining a diameter of 35 mm in 7 days at 25°C, plane, velvety, consisting of thin mycelial felt, producing abundant developing ascomata over the entire surface, margins thin and submerged, white in color; conidiogenesis lacking; reverse luteous (R12).

Colonies on OM showing the same colonial characteristic as on CM, attaining a diameter of 30 mm in 7 days at 25°C, plane, velvety, consisting of thin mycelial felt, producing abundant developing ascomata at the central colony, margins thin and submerged, white in color; conidiogenesis lacking; reverse luteous (R12).

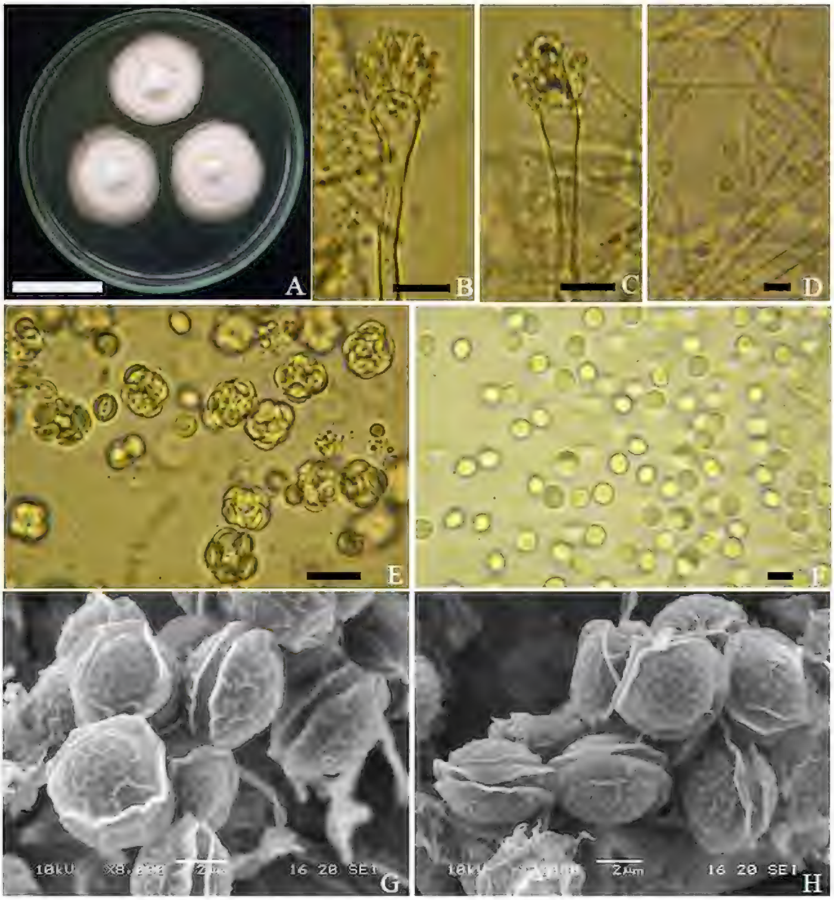

Fig. 1. *Aspergillus similanensis* (KUFA 0012): A. colony on MEA 25°C, 7d; B, C. conidial heads; D. conidia; E. asci; F. ascospores; G, H. ascospores (SEM). Scale bars: A = 3 cm; B, C, E = 10 µm, D, F = 5 µm.

Homothallic.

At 37°C, colonies on all media except CzA growing more rapidly than at 25°C, but no growth at 45°C.

Mycelium composed of branched, septate, smooth-walled, hyaline hyphae, 2–3 µm wide. Conidial heads small, short radiate, conidiophores 120–170 × 3–4 µm wide in the middle, thick and smooth-walled, frequently septate at basal area, vesicles subglobose, pyriform or subclavate 7–8 µm wide in diameter; uniseriate, phialides hyaline, 6–7 × 2–2.5 µm, covering the upper half of the vesicle, conidia pale green, globose to subglobose, 3–4 µm, smooth walled.

Ascomata non-ostiolate, superficial, globose, 120–150 µm, ripening within 10–14 days. Covering consisting of loosely hyaline, 4–5 µm wide, branched, septate, smooth-walled hyphae. Asci 8-spored, globose to subglobose, 10–11 µm, evanescent at maturity. Ascospores hyaline, lenticular, 5–5.5 µm including two low equatorial crests, 1–1.5 µm wide, spore body 4–4.5 µm and with the convex surfaces fine reticulate ornamentation.

Comments—The strain KUFA 0012 produced lenticular ascospores, convex surfaces decorated with fine reticulation with two low equatorial crests (Figs 1G–H). Ascospores of this species are similar to those of *A. huiyaniae* and *A. pernambucoensis,* which were recently reported from soils. In both of those species the convex surface ornamentation of the ascospores is irregularly ribbed to slightly reticulate. However, strain KUFA 0012 can be distinguished from the two other species by its smaller ascospore size as well as colony morphology on CzA in term of sporulation rate, colony coloration, and growth rate. Phylogenetic analysis of the β-tubulin gene comparing KUFA 0012 and 43 other strains for comparison (Matsuzawa et al. 2014b) and the calmodulin gene comparing KUFA 0012 with 10 related species revealed a close relationship with *Aspergillus huiyaniae* (Figs 2–3), while actin gene analysis placed KUFA 0012 in the same clade with *N. fennelliae* and *N. sublevispora* (Fig. 4). The β-tubulin, calmodulin, and actin DNA sequences were additionally analyzed using BLAST software (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The β-tubulin gene analysis showed KUFA 0012 closely related (98%) to *N. fennelliae*, and the calmodulin and actin gene analyses revealed a 97% of similarity with *N. fennelliae*.

Our phylogenetic analysis placed KUFA 0012 in a clade closely related to *A. huiyaniae, N. fennelliae,* and *N. sublevispora*. Despite its morphologically similar ascospores with *A. huiyaniae*, the KUFA 0012 strain produces smaller ascospores with two low equatorial crests. KUFA 0012 also differs from *N. sublevispora* in its distinct ascospore ornamentation presenting finely reticulate ascospores with two well separated crests instead of regularly microtuberculate ascospores with two closely appressed crests. Although the gene analysis revealed a close phylogenetic relationship with *N. fennelliae,* the strains are clearly separated by mating type: KUFA 0012 strain is homothallic and *N. fennelliae* was described as heterothallic.

Phylogenetic and morphological analyses showed that the marine-derived taxon is an undescribed species, which we have named *Aspergillus similanensis*. As recently reported by our research group (Prompanya et al. 2014, 2015), the ethyl acetate extract from KUFA 0013 contains seven new natural products—chevalone E; pyripyropene E and T; similanamide; and similanpyrone A, B,

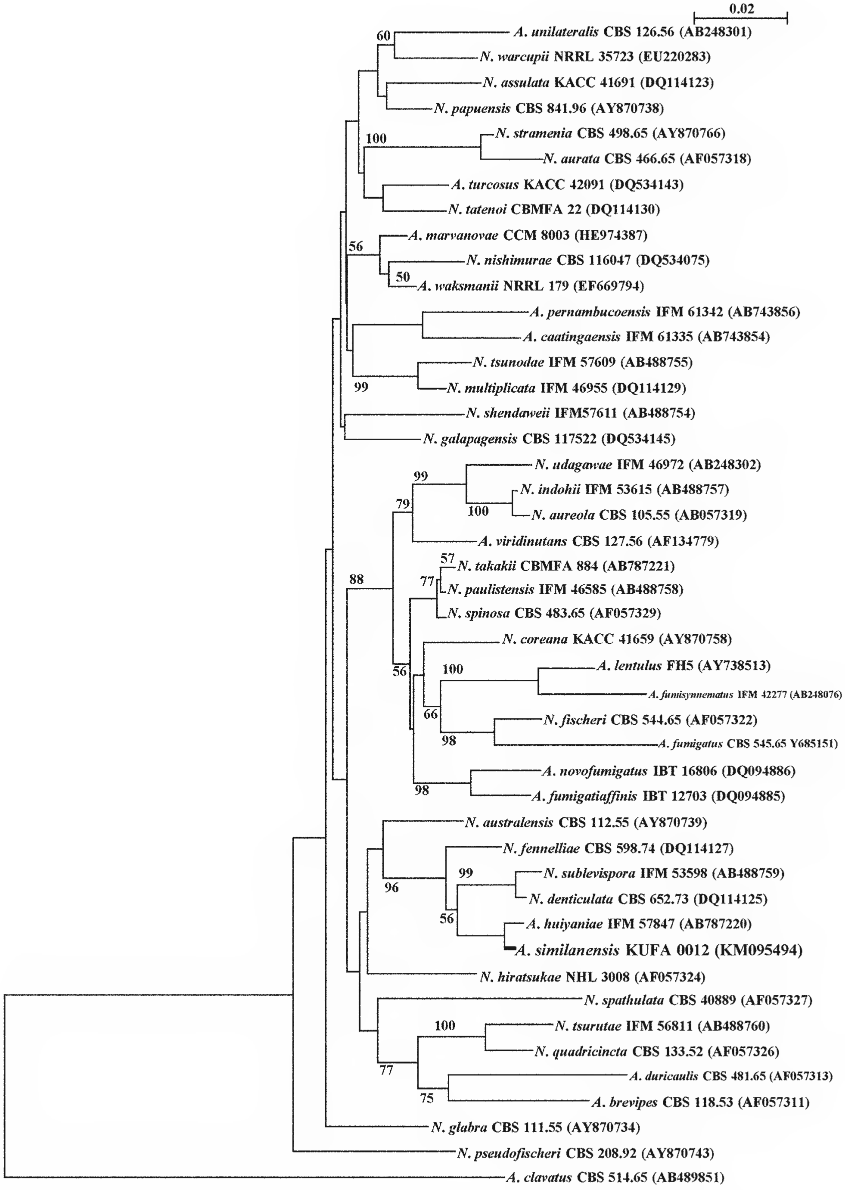
0.02
60
A. unilateralis CBS 126.56 (AB248301)
N. warcupii NRRL 35723 (EU220283)
N. assulata KACC 41691 (DQ114123)
N. papuensis CBS 841.96 (AY870738)
100
N. stramenia CBS 498.65 (AY870766)
N. aurata CBS 466.65 (AF057318)
A. turcosus KACC 42091 (DQ534143)
N. tatenoi CBMFA 22 (DQ114130)
56
A. marvanovae CCM 8003 (HE974387)
N. nishimurae CBS 116047 (DQ534075)
50
A. waksmanii NRRL 179 (EF669794)
A. pernambucoensis IFM 61342 (AB743856)
A. caatingaensis IFM 61335 (AB743854)
N. tsunodae IFM 57609 (AB488755)
99
N. multiplicata IFM 46955 (DQ114129)
N. shendaweii IFM57611 (AB488754)
N. galapagensis CBS 117522 (DQ534145)
N. udagawae IFM 46972 (AB248302)
99
N. indohii IFM 53615 (AB488757)
79
100
N. aureola CBS 105.55 (AB057319)
A. viridinutans CBS 127.56 (AF134779)
57
N. takakii CBMFA 884 (AB787221)
88
77
N. paulistensis IFM 46585 (AB488758)
N. spinosa CBS 483.65 (AF057329)
N. coreana KACC 41659 (AY870758)
100
A. lentulus FH5 (AY738513)
56
A. fumisynnematus IFM 42277 (AB248076)
66
N. fischeri CBS 544.65 (AF057322)
98
A. fumigatus CBS 545.65 Y685151)
A. novofumigatus IBT 16806 (DQ094886)
98
A. fumigatiaffinis IBT 12703 (DQ094885)
N. australensis CBS 112.55 (AY870739)
N. fennelliae CBS 598.74 (DQ114127)
99
N. sublevispora IFM 53598 (AB488759)
96
N. denticulata CBS 652.73 (DQ114125)
56
A. huiyaniae IFM 57847 (AB787220)
A. similanensis KUFA 0012 (KM095494)
N. hiratsukae NHL 3008 (AF057324)
N. spathulata CBS 40889 (AF057327)
100
N. tsurutae IFM 56811 (AB488760)
N. quadricincta CBS 133.52 (AF057326)
77
A. duricaulis CBS 481.65 (AF057313)
75
A. brevipes CBS 118.53 (AF057311)
N. glabra CBS 111.55 (AY870734)
N. pseudofischeri CBS 208.92 (AY870743)
A. clavatus CBS 514.65 (AB489851)

FIG. 2. Neighbor-joining tree from sequences of the β-tubulin gene. Numbers at nodes indicate the percentage of bootstrap sampling (>50%), derived from 1000 samples. *A.* = *Aspergillus*, *N.* = *Neosartorya.*

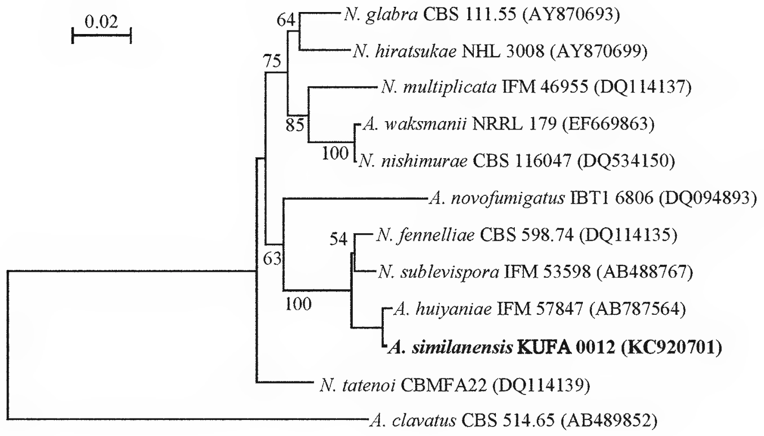
0.02
64
75
85
100
63
54
100
N. glabra CBS 111.55 (AY870693)
N. hiratsukae NHL 3008 (AY870699)
N. multiplicata IFM 46955 (DQ114137)
A. waksmanii NRRL 179 (EF669863)
N. nishimurae CBS 116047 (DQ534150)
A. novofumigatus IBT1 6806 (DQ094893)
N. fennelliae CBS 598.74 (DQ114135)
N. sublevispora IFM 53598 (AB488767)
A. huiyaniae IFM 57847 (AB787564)
A. similanensis KUFA 0012 (KC920701)
N. tatenoi CBMFA22 (DQ114139)
A. clavatus CBS 514.65 (AB489852)

FIG. 3. Neighbor-joining tree from sequences of the calmodulin gene. Numbers at nodes indicate the percentage of bootstrap sampling (>50%), derived from 1000 samples. *A.* = *Aspergillus*, *N.* = *Neosartorya*.

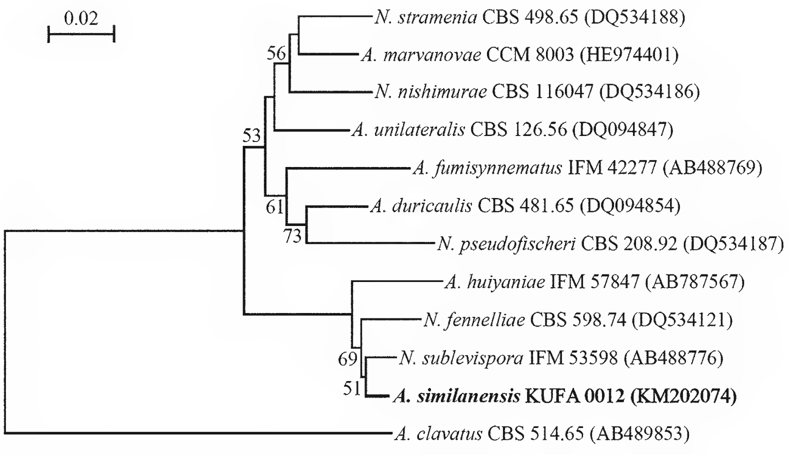
0.02
56
53
61
73
69
51
N. stramenia CBS 498.65 (DQ534188)
A. marvanovae CCM 8003 (HE974401)
N. nishimurae CBS 116047 (DQ534186)
A. unilateralis CBS 126.56 (DQ094847)
A. fumisynnematus IFM 42277 (AB488769)
A. duricaulis CBS 481.65 (DQ094854)
N. pseudofischeri CBS 208.92 (DQ534187)
A. huiyaniae IFM 57847 (AB787567)
N. fennelliae CBS 598.74 (DQ534121)
N. sublevispora IFM 53598 (AB488776)
A. similanensis KUFA 0012 (KM202074)
A. clavatus CBS 514.65 (AB489853)

FIG. 4. Neighbor-joining tree from sequences of the actin gene. Numbers at nodes indicate the percentage of bootstrap sampling (>50%), derived from 1000 samples. *A.* = *Aspergillus*, *N.* = *Neosartorya*.

and C—as well as seven previously described compounds. Interestingly, this new species was isolated from a marine sponge, an unusual habitat for teleomorphic states of *Aspergillus* sect. *Fumigati*, which have mainly been reported from soils and food (Eamvijarn et al. 2013a, Matsuzawa et al. 2014a,b). Despite the limited distribution in marine environments, we have previously reported several species of *Neosartorya* such as *N. paulistensis, N. laciniosa,* and *N. tsunodae* from marine sponges collected from Thai waters (Gomes et al. 2014). Chemical analysis of these species resulted in the isolation of several bioactive compounds (Eamvijarn et al. 2013a, Gomes et al. 2014). Since we have found only one strain of *A. similanensis*, we consider this species rare and presumably with limited distribution.

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